

PEPTIDES HAVING ANTIANGIOGENIC ACTIVITY

5

Technical Field

The present invention relates to novel compounds having activity useful for treating conditions which arise from or are exacerbated by angiogenesis, pharmaceutical compositions comprising the compounds, methods of treatment using the compounds, and
10 methods of inhibiting angiogenesis.

Background of the Invention

Angiogenesis is the fundamental process by which new blood vessels are formed and is essential to a variety of normal body activities (such as reproduction, development and wound repair). Although the process is not completely understood, it is believed to involve a complex interplay of molecules which both stimulate and inhibit the growth of endothelial cells, the primary cells of the capillary blood vessels. Under normal conditions these molecules appear to maintain the microvasculature in a quiescent state (i.e., one of no capillary growth) for prolonged periods that may last for weeks, or in some cases, decades.
15 However, when necessary, such as during wound repair, these same cells can undergo rapid proliferation and turnover within as little as five days (Folkman, J. and Shing, Y., *J. Biol. Chem.*, **267**(16): 10931-10934, and Folkman, J. and Klagsbrun, M., *Science*, **235**: 442-447 (1987)).
20

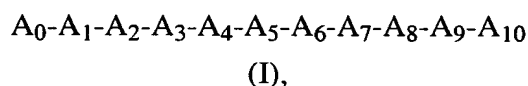
Although angiogenesis is a highly regulated process under normal conditions, many
25 diseases (characterized as "angiogenic diseases") are driven by persistent unregulated angiogenesis. Otherwise stated, unregulated angiogenesis may either cause a particular disease directly or exacerbate an existing pathological condition. For example, ocular neovascularization has been implicated as the most common cause of blindness. In certain existing conditions such as arthritis, newly formed capillary blood vessels invade the joints
30 and destroy cartilage. In diabetes, new capillaries formed in the retina invade the vitreous, bleed, and cause blindness. Growth and metastasis of solid tumors are also angiogenesis-dependent (Folkman, J., *Cancer Res.*, **46**: 467-473 (1986), Folkman, J., *J. Natl. Cancer Inst.*, **82**: 4-6 (1989)). It has been shown, for example, that tumors which enlarge to greater than 2 mm must obtain their own blood supply and do so by inducing the growth of new capillary
35 blood vessels. Once these new blood vessels become embedded in the tumor, they provide a means for tumor cells to enter the circulation and metastasize to distant sites, such as the liver, the lung, and the bones (Weidner, N., et. al., *N. Engl. J. Med.*, **324**(1): 1-8 (1991)).

Several angiogenesis inhibitors are currently under development for use in treating angiogenic diseases (Gasparini, G. and Harris, A.L., *J. Clin. Oncol.* **13**(3): 765-782, (1995)). A number of disadvantages have been associated with many of these compounds. A potent angiogenesis inhibitor, for example suramin, can cause severe systemic toxicity in humans at doses required to reach antitumor activity. Other compounds, such as retinoids, interferons, and antiestrogens are safe for human use, but have only a weak anti-angiogenic effect.

Peptides having angiogenesis inhibiting properties have been described in commonly-owned WO01/38397, WO01/38347, and WO99/61476. However, it would be desirable to prepare antiangiogenic compounds having improved profiles of activity.

Summary of the Invention

The present invention relates to a novel class of compounds having angiogenesis-inhibiting properties. The invention provides nona- and decapeptides with enhanced properties of angiogenesis inhibition. In its principle embodiment, the present invention provides a compound of formula (I)



or a therapeutically acceptable salt thereof, wherein

A₀ is absent or selected from the group consisting of N-acetyl, N-acetylazetidine-2-carbonyl, N-acetylazetidine-3-carbonyl, N-acetylnipecotyl, N-acetylpiperidine-4-acetyl, and N-acetylprolyl;

A₁ is selected from the group consisting of D-alanyl, (1R,3S)-1-aminocyclopentane-3-carbonyl, (1S,4R)-1-aminocyclopent-2-ene-4-carbonyl, 1-amino-1-cyclopropanecarbonyl, 3-(4-chlorophenyl)alanyl, 4-hydroxyprolyl, N-methylnorvalyl, 3-(4-methylphenyl)alanyl, N-methylprolyl, N-methylthreonyl(benzyl), norleucyl, propargylglycyl, sarcosyl, and (2,3,5,6-tetrahydro-1-thiopyran-4-yl)glycyl;

A₂ is selected from the group consisting of [(1S,3R)-1-aminocyclopentane-3-carbonyl], [(1R,4S)-1-aminocyclopent-2-ene-4-carbonyl], [(1S,4R)-1-aminocyclopent-2-ene-4-carbonyl], asparaginy, 3-(3-cyanophenyl)alanyl, 3-(4-cyanophenyl)alanyl, 3-(3,4-dimethoxyphenyl)alanyl, 3-(4-fluorophenyl)alanyl, 3-(2-furyl)alanyl, glutaminy, glycyl, 3-(4-methylphenyl)alanyl, norvalyl, and 3-(thiazol-5-yl)alanyl;

A₃ is selected from the group consisting of asparaginy, glutaminy, isoleucyl, and valyl;

A₄ is selected from the group consisting of D-alloisoleucyl, D-isoleucyl, D-leucyl, and D-penicillaminy(S-methyl);

A₅ is selected from the group consisting of allothreonyl, aspartyl, 4-hydroxyprolyl, seryl, threonyl, and threonyl(O-acetyl);

A₆ is selected from the group consisting of allothreonyl, glutaminy, 4-hydroxyprolyl, norvalyl, ornithyl(N-delta-acetyl), prolyl, seryl, and tryptyl;

A₇ is selected from the group consisting of isoleucyl, D-isoleucyl, and prolyl;

A₈ is selected from the group consisting of arginyl, glutaminy, and ornithyl;

A₉ is prolyl; and

A₁₀ is selected from the group consisting of D-alanylamide, D-lysyl(N-epsilon-acetyl)amide, ethylamide, and N-methyl-D-alanylamide;

provided that when A₀ is absent A₁ is N-methylprolyl; and

provided that when A₁ is sarcosyl A₀ is not acetyl; or A₂ is not asparaginy, glutaminy, or glycyl; or A₄ is not D-alloisoleucyl, D-isoleucyl, or D-leucyl; or A₅ is not allothreonyl, seryl, or threonyl; or A₆ is not glutaminy, norvalyl, seryl, or tryptyl; or A₈ is not arginyl; or A₁₀ is not D-alanylamide or ethylamide.

In another embodiment, the present invention provides a pharmaceutical composition comprising a compound of formula (I), or a therapeutically acceptable salt thereof, in combination with a therapeutically acceptable carrier.

In another embodiment, the present invention provides a method of inhibiting angiogenesis in a mammal in recognized need of such treatment comprising administering to the mammal a therapeutically acceptable amount of a compound of formula (I), or a therapeutically acceptable salt thereof.

Detailed Description of the Invention

As used herein, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise.

As used in the present specification the following terms have the meanings indicated:

The term "carbonyl," as used herein, represents -C(O)-.

The term "ethylamide," as used herein, represents -NHCH₂CH₃ at the C-terminus of an amino acid.

The term "nipecotyl," as used herein, represents the acyl group derived from nipecotic acid, i.e., piperidine-3-carboxylic acid.

The term "pharmaceutically acceptable salt," as used herein, represents salts or zwitterionic forms of the compounds of the present invention which are water or oil-soluble or dispersible, which are suitable for treatment of diseases without undue toxicity, irritation, and allergic response; which are commensurate with a reasonable benefit/risk ratio, and which are effective for their intended use. The salts can be prepared during the final isolation and purification of the compounds or separately by reacting an amino group with a suitable acid. Representative acid addition salts include acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate,

glycerophosphate, hemisulfate, heptanoate, hexanoate, formate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate (isethionate), lactate, maleate, mesitylenesulfonate, methanesulfonate, naphthylenesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, trichloroacetate, trifluoroacetate, phosphate, glutamate, bicarbonate, para-toluenesulfonate, and undecanoate. Also, amino groups in the compounds of the present invention can be quaternized with methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides; dimethyl, diethyl, dibutyl, and diamyl sulfates; decyl, lauryl, myristyl, and steryl chlorides, bromides, and iodides; and benzyl and phenethyl bromides. Examples of acids which can be employed to form therapeutically acceptable addition salts include inorganic acids such as hydrochloric, hydrobromic, sulfuric, and phosphoric, and organic acids such as oxalic, maleic, succinic, and citric.

Unless indicated otherwise by a "D-" prefix, *e.g.* D-Ala or N-Me-D-Ile, the stereochemistry of the α -carbon of the amino acids and aminoacyl residues in peptides described in this specification and the appended claims is the natural or "L" configuration. The Cahn-Ingold-Prelog "R" and "S" designations are used to specify the stereochemistry of chiral centers in certain acyl substituents at the N-terminus of the peptides of this invention. The designation "R,S" is meant to indicate a racemic mixture of the two enantiomeric forms. This nomenclature follows that described in R.S. Cahn, *et al.*, *Angew. Chem. Int. Ed. Engl.*, **5**, 385-415 (1966).

All peptide sequences are written according to the generally accepted convention whereby the α -N-terminal amino acid residue is on the left and the α -C-terminal is on the right. As used herein, the term " α -N-terminus" refers to the free α -amino group of an amino acid in a peptide, and the term " α -C-terminus" refers to the free α -carboxylic acid terminus of an amino acid in a peptide.

For the most part, the names on naturally occurring and non-naturally occurring aminoacyl residues used herein follow the naming conventions suggested by the IUPAC Commission on the Nomenclature of Organic Chemistry and the IUPAC-IUB Commission on Biochemical Nomenclature as set out in "Nomenclature of α -Amino Acids (Recommendations, 1974)" *Biochemistry*, **14**(2), (1975). To the extent that the names and abbreviations of amino acids and aminoacyl residues employed in this specification and appended claims differ from those suggestions, they will be made clear to the reader. Some abbreviations useful in describing the invention are defined below in the following Table 1.

Table 1

Abbreviation	Definition
N-Ac-Sar	N-acetylsarcosyl

Ala	alanyl
AlaNH ₂	alanylamide
N-Me-D-AlaNH ₂	N-methyl-D-alanylamide
alloIle	alloisoleucyl
alloThr	allothreonyl
alloThr(<i>t</i> -Bu)	allothreonyl(<i>O-t</i> -butyl)
Arg	arginyl
Arg(Pmc)	(N ^G -2,2,5,7,8-pentamethylchroman-6-sulfonyl)arginyl
Asn	asparaginy
Asn(Trt)	asparaginy(trityl)
Asp	aspartyl
Asp(<i>Ot</i> Bu)	aspartyl(<i>O-t</i> -butyl)
Fmoc	9-fluorenylmethoxycarbonyl
(2-furyl)Ala	3-(2-furyl)alanyl
Gln	glutaminyl
Gln(Trt)	glutaminyl(trityl)
Gly	glycyl
Hyp	4-hydroxyprolyl
Hyp(<i>Ot</i> Bu)	4-hydroxyprolyl(<i>O-t</i> -butyl)
Ile	isoleucyl
Leu	leucyl
Lys(Ac)NH ₂	lysyl(N-epsilon-acetyl)amide
Nle	norleucyl
Nva	norvalyl
Orn	ornithyl
Orn(N-delta-Ac)	ornithyl(N-delta-acetyl)
Orn(N-delta-Boc)	ornithyl(N-delta- <i>tert</i> -butoxycarbonyl)
Pen(SMe)	penicillaminy(S-methyl)
(4-Cl)Phe	3-(4-chlorophenyl)alanyl
(3-CN)Phe	3-(3-cyanophenyl)alanyl
(4-CN)Phe	3-(4-cyanophenyl)alanyl
(3,4-diMeO)Phe	3-(3,4-dimethoxyphenyl)alanyl
(4-F)Phe	3-(4-fluorophenyl)alanyl
(4-Me)Phe	3-(4-methylphenyl)alanyl
Pro	prolyl

ProNHCH ₂ CH ₃	prolylethylamide
N-MePro	N-methylprolyl
PropargylGly	propargylglycyl
Sar	sarcosyl
Ser	seryl
Ser(OBzl)	seryl(O-benzyl)
Ser(O t Bu)	seryl(O- t -butyl)
Taz	3-(thiazol-5-yl)alanyl
Thr	threonyl
Thr(OBzl)	threonyl(O-benzyl)
Thr(O t Bu)	threonyl(O- t -butyl)
Thr(OAc)	threonyl(O-acetyl)
N-MeThr(OBzl)	N-methylthreonyl(O-benzyl)
Val	valyl

When not found in the table above, nomenclature and abbreviations may be further clarified by reference to the Calbiochem-Novabiochem Corp. *1999 Catalog and Peptide Synthesis Handbook* or the Chem-Impex International, Inc. *Tools for Peptide & Solid Phase Synthesis 1998-1999 Catalogue*.

Compositions

The compounds of the invention, including not limited to those specified in the examples, possess anti-angiogenic activity. As angiogenesis inhibitors, such compounds are useful in the treatment of both primary and metastatic solid tumors, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma) and tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas). Such compounds may also be useful in treating solid tumors arising from hematopoietic malignancies such as leukemias (i.e. chloromas, plasmacytomas and the plaques and tumors of mycosis fungoides and cutaneous T-cell lymphoma/leukemia) as well as in the treatment

of lymphomas (both Hodgkin's and non-Hodgkin's lymphomas). In addition, these compounds may be useful in the prevention of metastases from the tumors described above either when used alone or in combination with radiotherapy and/or other chemotherapeutic agents.

Further uses include the treatment and prophylaxis of autoimmune diseases such as rheumatoid, immune and degenerative arthritis; various ocular diseases such as diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, retrolental fibroplasia, neovascular glaucoma, rubeosis, retinal neovascularization due to macular degeneration, hypoxia, angiogenesis in the eye associated with infection or surgical intervention, and other abnormal neovascularization conditions of the eye; skin diseases such as psoriasis; blood vessel diseases such as hemangiomas, and capillary proliferation within atherosclerotic plaques; Osler-Webber Syndrome; myocardial angiogenesis; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; and wound granulation. Other uses include the treatment of diseases characterized by excessive or abnormal stimulation of endothelial cells, including not limited to intestinal adhesions, Crohn's disease, atherosclerosis, scleroderma, and hypertrophic scars, i.e. keloids. Another use is as a birth control agent, by inhibiting ovulation and establishment of the placenta. The compounds of the invention are also useful in the treatment of diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (*Rochela minutesalia quintosa*) and ulcers (*Helicobacter pylori*). The compounds of the invention are also useful to reduce bleeding by administration prior to surgery, especially for the treatment of resectable tumors.

The compounds of the invention may be used in combination with other compositions and procedures for the treatment of diseases. For example, a tumor may be treated conventionally with surgery, radiation or chemotherapy combined with a peptide of the present invention and then a peptide of the present invention may be subsequently administered to the patient to extend the dormancy of micrometastases and to stabilize and inhibit the growth of any residual primary tumor. Additionally, the compounds of the invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions.

A sustained-release matrix, as used herein, is a matrix made of materials, usually polymers, which are degradable by enzymatic or acid-base hydrolysis or by dissolution. Once inserted into the body, the matrix is acted upon by enzymes and body fluids. A sustained-release matrix desirably is chosen from biocompatible materials such as liposomes, polylactides (polylactic acid), polyglycolide (polymer of glycolic acid), polylactide co-glycolide (copolymers of lactic acid and glycolic acid) polyanhydrides, poly(ortho)esters, polypeptides, hyaluronic acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids,

phospholipids, polysaccharides, nucleic acids, polyamino acids, amino acids such as phenylalanine, tyrosine, isoleucine, polynucleotides, polyvinyl propylene, polyvinylpyrrolidone and silicone. A preferred biodegradable matrix is a matrix of one of either polylactide, polyglycolide, or polylactide co-glycolide (co-polymers of lactic acid and glycolic acid).

When used in the above or other treatments, a therapeutically effective amount of one of the compounds of the present invention may be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt form. By a "therapeutically effective amount" of the compound of the invention is meant a sufficient amount of the compound to treat an angiogenic disease, (for example, to limit tumor growth or to slow or block tumor metastasis) at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

Alternatively, a compound of the present invention may be administered as pharmaceutical compositions containing the compound of interest in combination with one or more pharmaceutically acceptable excipients. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The compositions may be administered parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), rectally, or buccally. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Pharmaceutical compositions for parenteral injection comprise pharmaceutically-acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene

glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity may be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

5 These compositions may also contain adjuvants such as preservative, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the
10 injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters), poly(anhydrides), and (poly)glycols, such as PEG. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

The injectable formulations may be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Topical administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for topical administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized.
25 In non-pressurized powder compositions, the active ingredient in finely divided form may be used in admixture with a larger-sized pharmaceutically-acceptable inert carrier comprising particles having a size, for example, of up to 100 micrometers in diameter. Suitable inert carriers include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers.

30 Alternatively, the composition may be pressurized and contain a compressed gas, such as nitrogen or a liquified gas propellant. The liquified propellant medium and indeed the total composition is preferably such that the active ingredient does not dissolve therein to any substantial extent. The pressurized composition may also contain a surface active agent, such as a liquid or solid non-ionic surface active agent or may be a solid anionic surface
35 active agent. It is preferred to use the solid anionic surface active agent in the form of a sodium salt.

5 A further form of topical administration is to the eye. A compound of the invention is delivered in a pharmaceutically acceptable ophthalmic vehicle, such that the compound is maintained in contact with the ocular surface for a sufficient time period to allow the compound to penetrate the corneal and internal regions of the eye, as for example the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid/retina and sclera. The pharmaceutically-acceptable ophthalmic vehicle may, for example, be an ointment, vegetable oil or an encapsulating material. Alternatively, the compounds of the invention may be injected directly into the vitreous and aqueous humour.

10 Compositions for rectal or vaginal administration are preferably suppositories which may be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at room temperature liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

15 Compounds of the present invention may also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically-acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*

20 While the compounds of the invention can be administered as the sole active pharmaceutical agent, they may also be used in combination with one or more agents which are conventionally administered to patients for treating angiogenic diseases. For example, the compounds of the invention are effective over the short term to make tumors more sensitive to traditional cytotoxic therapies such as chemicals and radiation. The compounds of the invention also enhance the effectiveness of existing cytotoxic adjuvant anti-cancer therapies. The compounds of the invention may also be combined with other antiangiogenic agents to enhance their effectiveness, or combined with other antiangiogenic agents and administered together with other cytotoxic agents. In particular, when used in the treatment of solid tumors, compounds of the invention may be administered with IL-12, retinoids, interferons, angiostatin, endostatin, thalidomide, thrombospondin-1, thrombospondin-2, captopryl, angiostatin, TNP-470, pentosan polysulfate, platelet factor 4, LM-609, SU-5416, CM-101, Tecogalan, plasminogen-K-5, vasostatin, vitaxin, vasculostatin, squalamine,

marimastat or other MMP inhibitors, anti-neoplastic agents such as alpha inteferon, COMP (cyclophosphamide, vincristine, methotrexate and prednisone), etoposide, mBACOD (methortrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone), PRO-MACE/MOPP (prednisone, methotrexate (w/leucovin rescue), doxorubicin, cyclophosphamide, cisplatin, taxol, etoposide/mechllorethamine, vincristine, prednisone and procarbazine), vincristine, vinblastine, and the like as well as with radiation.

Total daily dose of the compositions of the invention to be administered to a human or other mammal host in single or divided doses may be in amounts, for example, from 0.0001 to 300 mg/kg body weight daily and more usually 1 to 300 mg/kg body weight.

It will be understood that agents which can be combined with the compound of the present invention for the inhibition, treatment or prophylaxis of angiogenic diseases are not limited to those listed above, include in principle any agents useful for the treatment or prophylaxis of angiogenic diseases.

Determination of Biological Activity

In Vitro Assay for Angiogenic Activity

The human microvascular endothelial (HMVEC) migration assay was run according to the procedure of S. S. Tolsma, O. V. Volpert, D. J. Good, W. F. Frazier, P. J. Polverini and N. Bouck, J. Cell Biol. 122, 497-511 (1993).

The HMVEC migration assay was carried out using Human Microvascular Endothelial Cells-Dermal (single donor) and Human Microvascular Endothelial Cells, (neonatal). The BCE or HMVEC cells were starved overnight in DME containing 0.01% bovine serum albumin (BSA). Cells were then harvested with trypsin and resuspended in DME with 0.01% BSA at a concentration of 1.5×10^6 cells per mL. Cells were added to the bottom of a 48 well modified Boyden chamber (Nucleopore Corporation, Cabin John, MD). The chamber was assembled and inverted, and cells were allowed to attach for 2 hours at 37 °C to polycarbonate chemotaxis membranes (5 µm pore size) that had been soaked in 0.01% gelatin overnight and dried. The chamber was then reinverted, and test substances (total volume of 50 µL), including activators, 15 ng/mL bFGF/VEGF, were added to the wells of the upper chamber. The apparatus was incubated for 4 hours at 37 °C. Membranes were recovered, fixed and stained (Diff Quick, Fisher Scientific) and the number of cells that had migrated to the upper chamber per 3 high power fields counted. Background migration to DME + 0.1 BSA was subtracted and the data reported as the number of cells migrated per 10 high power fields (400X) or, when results from multiple experiments were combined, as the percent inhibition of migration compared to a positive control.

Representative compounds described in Examples 1 to 64 inhibited human endothelial cell migration in the above assay by at least 50% inhibition when tested at a concentration of 100 nM. Preferred compounds inhibited human endothelial cell migration by 63-74 percent when tested at a concentration of 100 nM. More preferred compounds inhibited human endothelial cell migration by 61-97 percent at a concentration of 1 nM, and the most preferred compounds inhibited human endothelial cell migration by 80-86 percent at a concentration of 0.1 nM. As shown by these results, the compounds of the present invention demonstrate enhanced potency.

Synthesis of the Peptides

This invention is intended to encompass compounds having formula (I) when prepared by synthetic processes or by metabolic processes. Preparation of the compounds of the invention by metabolic processes include those occurring in the human or animal body (*in vivo*) or processes occurring *in vitro*.

The polypeptides of the present invention may be synthesized by many techniques that are known to those skilled in the art. For solid phase peptide synthesis, a summary of the many techniques may be found in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, W.H. Freeman Co. (San Francisco), 1963 and J. Meienhofer, Hormonal Proteins and Peptides, vol. 2, p. 46, Academic Press (New York), 1973. For classical solution synthesis see G. Schroder and K. Lupke, The Peptides, vol. 1, Academic Press (New York), 1965.

Reagents, resins, amino acids, and amino acid derivatives are commercially available and can be purchased from Chem-Impex International, Inc. (Wood Dale, IL, U.S.A.) or Calbiochem-Novabiochem Corp. (San Diego, CA, U.S.A.) unless otherwise noted herein.

In general, these methods comprise the sequential addition of one or more amino acids or suitably protected amino acids to a growing peptide chain. Normally, either the amino or carboxy group of the first amino acid is protected by a suitable protecting group. The protected or derivatized amino acid can then be either attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence having the complementary (amino or carboxy) group suitably protected, under conditions suitable for forming the amide linkage. The protecting group is then removed from this newly added amino acid residue and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining protecting groups (and any solid support) are removed sequentially or concurrently, to afford the final polypeptide. By simple modification of this general procedure, it is possible to add more than one amino acid at a time to a growing chain, for example, by coupling (under

conditions which do not racemize chiral centers) a protected tripeptide with a properly protected dipeptide to form, after deprotection, a pentapeptide.

A particularly preferred method of preparing compounds of the present invention involves solid phase peptide synthesis. In this particularly preferred method the α -amino function is protected by an acid or base sensitive group. Such protecting groups should have the properties of being stable to the conditions of peptide linkage formation, while being readily removable without destruction of the growing peptide chain or racemization of any of the chiral centers contained therein. Suitable protecting groups are 9-fluorenylmethyloxycarbonyl (Fmoc), *t*-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), biphenylisopropyl-oxycarbonyl, *t*-amyloxycarbonyl, isobornyloxycarbonyl, (α,α)-dimethyl-3,5-dimethoxybenzyloxycarbonyl, *O*-nitrophenylsulfenyl, 2-cyano-*t*-butyloxycarbonyl, and the like. The 9-fluorenylmethyloxycarbonyl (Fmoc) protecting group is preferred.

Particularly preferred side chain protecting groups are: for arginine: acetyl (Ac), adamantyloxycarbonyl, benzyloxycarbonyl (Cbz), *t*-butyloxycarbonyl (Boc), 4-methoxybenzenesulfonyl, N^G-4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr), nitro, 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc), and *p*-toluenesulfonyl; for asparagine: (Trt); for aspartyl: *t*-butyl (*t*Bu); for glutaminyl: trityl (Trt); for ornithine: *t*-butoxycarbonyl (Boc); for penicillamine: methyl; for serine: *t*-butyl (*t*Bu), benzyl (Bzl), and tetrahydropyranyl; and for threonine: acetyl (Ac), benzyl, and *t*-butyl (*t*Bu).

In the solid phase peptide synthesis method, the C-terminal amino acid is attached to a suitable solid support or resin. Suitable solid supports useful for the above synthesis are those materials which are inert to the reagents and reaction conditions of the stepwise condensation-deprotection reactions, as well as being insoluble in the media used. The preferred solid support for synthesis of C-terminal carboxy peptides is 4-hydroxymethylphenoxymethyl-copoly(styrene-1% divinylbenzene). The preferred solid support for C-terminal amide peptides is 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)phenoxy-acetamidoethyl resin available from Applied Biosystems.

The C-terminal amino acid is coupled to the resin by means of a coupling mediated by N,N'-dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide (DIC), [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] (HATU), or *O*-benzotriazol-1-yl-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU), with or without 4-dimethylaminopyridine (DMAP), 1-hydroxybenzotriazole (HOBT), N-methylmorpholine (NMM), benzotriazol-1-yloxy-tris(dimethylamino)phosphonium-hexafluorophosphate (BOP) or bis(2-oxo-3-oxazolidinyl)phosphine chloride (BOPCl), for about 1 to about 24 hours at a temperature of between 10 °C and 50 °C in a solvent such as dichloromethane or DMF.

When the solid support is 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamidoethyl resin, the Fmoc group is cleaved with a secondary amine, preferably piperidine, prior to coupling with the C-terminal amino acid as described above. The preferred reagents used in the coupling to the deprotected 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)phenoxyacetamidoethyl resin are *O*-benzotriazol-1-yl-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU, 1 equiv.) and 1-hydroxybenzotriazole (HOBT, 1 equiv.), or [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] (HATU, 1 equiv.), in DMF.

The coupling of successive protected amino acids can be carried out in an automatic polypeptide synthesizer as is well known in the art. In a preferred embodiment, the α -amino function in the amino acids of the growing peptide chain are protected with Fmoc. The removal of the Fmoc protecting group from the N-terminal side of the growing peptide is accomplished by treatment with a secondary amine, preferably piperidine. Each protected amino acid is then introduced in about 3-fold molar excess and the coupling is preferably carried out in DMF. The coupling agent is normally *O*-benzotriazol-1-yl-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU, 1 equiv.) and 1-hydroxy-benzotriazole (HOBT, 1 equiv.) or [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] (HATU, 1 equiv.).

At the end of the solid phase synthesis, the polypeptide is removed from the resin and deprotected, either in succession or in a single operation. Removal of the polypeptide and deprotection can be accomplished in a single operation by treating the resin-bound polypeptide with a cleavage reagent, for example trifluoroacetic acid containing thianisole, water, or ethanedithiol.

In cases where the C-terminus of the polypeptide is an alkylamide, the resin is cleaved by aminolysis with an alkylamine. Alternatively, the peptide may be removed by transesterification, e.g. with methanol, followed by aminolysis or by direct transamidation. The protected peptide may be purified at this point or taken to the next step directly. The removal of the side chain protecting groups is accomplished using the cleavage cocktail described above.

The fully deprotected peptide is purified by a sequence of chromatographic steps employing any or all of the following types: ion exchange on a weakly basic resin in the acetate form; hydrophobic adsorption chromatography on underivitized polystyrene-divinylbenzene (for example, AMBERLITE[®] XAD); silica gel adsorption chromatography; ion exchange chromatography on carboxymethylcellulose; partition chromatography, e.g. on SEPHADEX[®] G-25, LH-20 or countercurrent distribution; high performance liquid chromatography (HPLC), especially reverse-phase HPLC on octyl- or octadecylsilyl-silica bonded phase column packing.

The foregoing may be better understood in light of the examples which are meant to describe compounds and process which can be carried out in accordance with the invention and are not intended as a limitation on the scope of the invention in any way.

Abbreviations which have been used the following examples are: DMF for N,N-dimethylformamide; HBTU for *O*-benzotriazol-1-yl-N,N,N',N'-tetramethyluroniumhexafluorophosphate; NMM for N-methylmorpholine; TFA for trifluoroacetic acid; NMP for N-methylpyrrolidinone; and HATU for [O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate].

EXAMPLE 1

N-(N-acetyl nipecotyl)-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

In the reaction vessel of a Rainin peptide synthesizer was placed Fmoc-Pro-Sieber ethylamide resin (0.25 g, 0.4 mmol/g loading) resin. The resin was solvated with DMF and amino acids were coupled sequentially according to the following synthetic cycle:

- (1) Resin solvated with DMF for about 5 minutes;
- (2) Resin washed 3 times with DMF for 1.5 minutes each time;
- (3) Fmoc group removed using 20% piperidine solution in DMF for 15 minutes, resin washed, and the sequence repeated;
- (4) Resin washed 6 times with DMF for 3 minutes each time;
- (5) Amino acid added;
- (6) Amino acid activated with 0.4M HBTU/NMM and coupled;
- (7) Resin washed 3 times with DMF for 1.5 minutes each time.

The protected amino acids were coupled to the resin in the following order:

Amino Acid	Coupling time
1. Fmoc-Arg(Pmc)	30 minutes
2. Fmoc-Ile	30 minutes
3. Fmoc-Nva	30 minutes
4. Fmoc-Thr(O ⁱ Bu)	30 minutes
5. Fmoc-D-Ile	30 minutes
6. Fmoc-Val	30 minutes
7. Fmoc-Gly	30 minutes
8. Fmoc-Sar	30 minutes
9. N-acetyl nipecotic acid	30 minutes

Upon completion of the synthesis the peptide was cleaved from the resin using a mixture of (95:2.5:2.5) TFA/anisole/water for 3 hours. The peptide solution was

concentrated *in vacuo*, precipitated with diethyl ether, and filtered. The crude peptide was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-(N-acetyl nipecotyl)-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-

ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.36 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1105 (M+H)⁺; Amino Acid Anal.: 0.92 Sar; 1.02 Gly; 1.00 Val; 2.10 Ile; 0.47 Thr; 0.93 Nva; 1.10 Arg; 1.06 Pro.

EXAMPLE 2

N-[N-acetylpiperidine-4-acetyl]-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-acetylpiperidine-4-acetic acid for N-acetyl nipecotic acid in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-[N-acetylpiperidine-4-acetyl]-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.32 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1119 (M+H)⁺; Amino Acid Anal.: 1.03 Sar; 0.97 Gly; 0.98 Val; 2.04 Ile; 0.51 Thr; 0.89 Nva; 1.06 Arg; 1.03 Pro.

EXAMPLE 3

N-Ac-Pro-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-acetylproline for N-acetyl nipecotic acid in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Pro-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.34 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1091 (M+H)⁺; Amino Acid Anal.: 0.90 Sar; 0.96 Gly; 0.99 Val; 2.07 Ile; 0.48 Thr; 1.01 Nva; 1.08 Arg; 2.12 Pro.

EXAMPLE 4

N-Ac-Sar-(4-CN)Phe-Val-D-alloIle-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid, Fmoc-(4-CN)Phe for Fmoc-Gly and Fmoc-D-alloIle for Fmoc-D-Ile in Example 1.

Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-(4-CN)Phe-Val-D-alloIle-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 4.74 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1109 (M+H)⁺; Amino Acid Anal.: 0.94 Sar; 1.02 Val; 2.13 Ile; 0.39 Thr; 0.94 Nva; 1.33 Arg; 1.04 Pro.

EXAMPLE 5

N-Ac-Sar-Gly-Val-D-Ile-Asp-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid and Fmoc-Asp(OtBu) for Fmoc-Thr(OtBu) in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-Gly-Val-D-Ile-Asp-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 2.80 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1008 (M+H)⁺; Amino Acid Anal.: 1.01 Sar; 1.02 Gly; 0.93 Val; 2.07 Ile; 0.88 Asp; 1.03 Nva; 1.37 Arg; 1.05 Pro.

EXAMPLE 6

N-Ac-Sar-Taz-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid and Fmoc-Taz for Fmoc-Gly in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-Taz-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t =

3.933 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1091 (M^+).

5

EXAMPLE 7

N-Ac-Sar-(3,4-diMeO)Phe-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid and Fmoc-(3,4-diMeO)Phe for Fmoc-Gly in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-(3,4-diMeO)Phe-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 4.27 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1144 (M^+).

15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
185
190
195
200
205
210
215
220
225
230
235
240
245
250
255
260
265
270
275
280
285
290
295
300
305
310
315
320
325
330
335
340
345
350
355
360
365
370
375
380
385
390
395
400
405
410
415
420
425
430
435
440
445
450
455
460
465
470
475
480
485
490
495
500
505
510
515
520
525
530
535
540
545
550
555
560
565
570
575
580
585
590
595
600
605
610
615
620
625
630
635
640
645
650
655
660
665
670
675
680
685
690
695
700
705
710
715
720
725
730
735
740
745
750
755
760
765
770
775
780
785
790
795
800
805
810
815
820
825
830
835
840
845
850
855
860
865
870
875
880
885
890
895
900
905
910
915
920
925
930
935
940
945
950
955
960
965
970
975
980
985
990
995

EXAMPLE 8

N-Ac-Sar-(2-furyl)Ala-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid and Fmoc-3-(2-furyl)Ala for Fmoc-Gly. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-(2-furyl)Ala-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 4.50 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1074 (M^+).

30

EXAMPLE 9

N-Ac-Sar-[(1S,3R)-1-aminocyclopentane-3-carbonyl]-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid and (1S,3R)-N-Fmoc-1-aminocyclopentane-3-carboxylic acid for Fmoc-Gly in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was

obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-[(1S,3R)-1-aminocyclopentane-3-carbonyl]-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.916 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1048 (M⁺).

EXAMPLE 10

N-Ac-Sar-[(1R,4S)-1-aminocyclopent-2-ene-4-carbonyl]-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid and (1R,4S)-N-Fmoc-1-aminocyclopent-2-ene-4-carboxylic acid for Fmoc-Gly in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-[(1R,4S)-1-aminocyclopent-2-ene-4-carbonyl]-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.918 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1046 (M⁺).

EXAMPLE 11

N-Ac-Sar-[(1S,4R)-1-aminocyclopent-2-ene-4-carbonyl]-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid and (1S,4R)-N-Fmoc-1-aminocyclopent-2-ene-4-carboxylic acid for Fmoc-Gly in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-[(1S,4R)-1-aminocyclopent-2-ene-4-carbonyl]-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.892 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1046 (M⁺).

EXAMPLE 12

N-Ac-Sar-(3-CN)Phe-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid, Fmoc-(3-CN)Phe for Fmoc-Gly, and Fmoc-D-Leu for Fmoc-D-Ile in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-(3-CN)Phe-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: $R_t = 4.636$ minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1109 (M^+).

EXAMPLE 13

N-Ac-Sar-(4-F)Phe-Val-D-alloIle-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid, Fmoc-(4-F)Phe for Fmoc-Gly, and Fmoc-D-alloIle for Fmoc-D-Ile in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-(4-F)Phe-Val-D-alloIle-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: $R_t = 4.778$ minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1102 (M^+).

EXAMPLE 14

N-Ac-Sar-(4-Me)Phe-Val-D-alloIle-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid, Fmoc-(4-Me)Phe for Fmoc-Gly, and Fmoc-D-alloIle for Fmoc-D-Ile in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-(4-Me)Phe-Val-D-alloIle-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: $R_t = 4.978$ minutes (using a C-18 column

and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1098 (M^+).

EXAMPLE 15

N-[(1S,4R)-1-N-acetylaminocyclopent-2-ene-4-carbonyl]-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetyl nipecotic acid, (1S,4R)-N-Fmoc-1-N-aminocyclopent-2-ene-4-carboxylic acid for Fmoc-Sar, and Fmoc-D-Leu for Fmoc-D-Ile in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-[(1S,4R)-1-N-acetylaminocyclopent-2-ene-4-carbonyl]-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.823 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1031 (M^+).

EXAMPLE 16

N-[(1R,3S)-1-N-acetylaminocyclopentane-3-carbonyl]-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetyl nipecotic acid, (1R,3S)-N-Fmoc-1-aminocyclopentane-3-carboxylic acid for Fmoc-Sar, and D-Leu for Fmoc-D-Ile in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-[(1R,3S)-1-N-acetylaminocyclopentane-3-carbonyl]-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.804 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1033 (M^+).

EXAMPLE 17

N-Ac-(4-Me)Phe-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetyl nipecotinic acid, Fmoc-(4-Me)Phe for Fmoc-Sar, and D-Leu for Fmoc-D-Ile in Example 1. Upon

completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-(4-Me)Phe-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.888 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1084 (M+H)⁺.

EXAMPLE 19

N-(N-acetyl-1-amino-1-cyclopropanecarbonyl)-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-1-amino-1-cyclopropylcarboxylic acid for Fmoc-Sar, and Fmoc-D-Leu for Fmoc-D-Ile in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-(N-acetyl-1-amino-1-cyclopropylcarbonyl)-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.888 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1005 (M⁺).

EXAMPLE 20

N-Ac-(2,3,5,6-Tetrahydro-1-thiopyran-4-yl)gly-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-(2,3,5,6-Tetrahydro-1-thiopyran-4-yl)gly for Fmoc-Sar, and Fmoc-D-Leu for Fmoc-D-Ile in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-(2,3,5,6-tetrahydro-1-thiopyran-4-yl)gly-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 4.464 minutes (using a C-18 column and a solvent system increasing in gradient

over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1079 (M^+).

EXAMPLE 21

N-Ac-Hyp-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-Hyp(OtBu) for Fmoc-Sar, and Fmoc-D-Leu for Fmoc-D-Ile in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Hyp-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 4.148 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1035 (M^+).

EXAMPLE 22

N-Ac-Nle-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-Nle for Fmoc-Sar, and Fmoc-D-Leu for Fmoc-D-Ile in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Nle-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 4.671 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1036 (M^+).

EXAMPLE 23

N-Ac-(4-Cl)Phe-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-(4-Cl)Phe for Fmoc-Sar, and Fmoc-D-Leu for Fmoc-D-Ile in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions

were lyophilized to provide N-Ac-(4-Cl)Phe-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 4.918 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1103 (M⁺).

5

EXAMPLE 24

N-Ac-propargylgly-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-Propargylgly for Fmoc-Sar, and Fmoc-D-Leu for Fmoc-D-Ile in Example 1.

10 Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Propargylgly-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 4.02 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1017 (M⁺).

0915956
10915956

EXAMPLE 25

N-Ac-D-Ala-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-D-Ala for Fmoc-Sar, and Fmoc-D-Leu for Fmoc-D-Ile in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This

25 was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-D-Ala-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.765 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 993 (M⁺).

30

EXAMPLE 26

N-Ac-Sar-Gly-Val-D-Ile-alloThr-Pro-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid, Fmoc-alloThr(OtBu) for Fmoc-Thr(OtBu), and Fmoc-Pro for Fmoc-Nva in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was

35

obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-Gly-Val-D-Ile-alloThr-Pro-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 3.551 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 992 (M+H)⁺.

EXAMPLE 27

N-Ac-Sar-Nva-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid and Fmoc-Nva for Fmoc-Gly in Example 1. After cleavage of the peptide from the resin and removal of the protecting groups the product was precipitated with diethyl ether and filtered. The product was purified by preparative HPLC to provide N-Ac-Sar-Nva-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 3.57 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 95% acetonitrile/water containing 10 mM ammonium acetate); MS (ESI) m/e 1036 (M+H)⁺; Amino Acid Anal.: 0.98 Sar; 2.02 Nva; 1.02 Val; 2.07 Ile; 0.51 Thr; 1.44 Arg; 1.04 Pro.

EXAMPLE 28

N-Ac-Sar-Asn-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid and Fmoc-Asn(Trt) for Fmoc-Gly in Example 1. After cleavage of the peptide from the resin and removal of the protecting groups the product was precipitated with diethyl ether and filtered. The product was purified by preparative HPLC to provide N-Ac-Sar-Asn-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 3.01 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 95% acetonitrile/water containing 10 mM ammonium acetate); MS (ESI) m/e 1051 (M+H)⁺; Amino Acid Anal.: 0.96 Sar; 1.01 Asn; 1.03 Val; 1.01 Nva; 1.03 Val; 2.12 Ile; 0.48 Thr; 1.32 Arg; 1.07 Pro.

EXAMPLE 29

N-Ac-Sar-Gly-Val-D-alloIle-Hyp-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-D-alloIle for Fmoc-D-Ile, and Fmoc-Hyp(OrBu) for Fmoc-Thr(OrBu) in Example 1. After cleavage of the peptide from the resin and removal of the protecting groups the product was precipitated with diethyl ether and filtered. The product was purified by preparative HPLC to provide N-Ac-Sar-Gly-Val-D-alloIle-Hyp-Nva-Ile-Arg-ProNHCH₂CH₃

as the trifluoroacetate salt; $R_t = 3.08$ minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 95% acetonitrile/water containing 10 mM ammonium acetate); MS (ESI) m/e 1051 ($M+H$)⁺.

5

EXAMPLE 30

N-Ac-Sar-Gly-Val-D-alloIle-Thr-Hyp-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-D-alloIle for Fmoc-D-Ile, and Fmoc-Hyp(O*t*Bu) for Fmoc-Nva in Example 1. After cleavage of the peptide from the resin and removal of the protecting groups the product was precipitated with diethyl ether and filtered. The product was purified by preparative HPLC to provide N-Ac-Sar-Gly-Val-D-alloIle-Thr-Hyp-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; $R_t = 2.71$ minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 95% acetonitrile/water containing 10 mM ammonium acetate); MS (ESI) m/e 1008 ($M+H$)⁺.

10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
280
290
300
310
320
330
340
350
360
370
380
390
400
410
420
430
440
450
460
470
480
490
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
660
670
680
690
700
710
720
730
740
750
760
770
780
790
800
810
820
830
840
850
860
870
880
890
900
910
920
930
940
950
960
970
980
990
1000

EXAMPLE 31

N-Ac-Sar-Gly-Val-D-Pen(SMe)-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid and Fmoc-D-Pen(SMe) for Fmoc-D-Ile in Example 1. After cleavage of the peptide from the resin and removal of the protecting groups the product was precipitated with diethyl ether and filtered. The product was purified by preparative HPLC to provide N-Ac-Sar-Gly-Val-D-Pen(SMe)-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; $R_t = 24.0$ minutes (using a C-18 column and a solvent system increasing in gradient over 33 minutes from 10% to 40% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1026 ($M+H$)⁺.

25

EXAMPLE 32

N-Ac-Sar-Gly-Val-D-Pen(SMe)-Ser-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-D-Pen(SMe) for Fmoc-D-Ile, and Fmoc-Ser(O*t*Bu) for Fmoc-Thr(O*t*Bu) in Example 1. After cleavage of the peptide from the resin and removal of the protecting groups the product was precipitated with diethyl ether and filtered. The product was purified by preparative HPLC to provide N-Ac-Sar-Gly-Val-D-Pen(SMe)-Ser-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; $R_t = 20.5$ minutes (using a C-18 column and a solvent system increasing in gradient over 33 minutes from 10% to 40% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1012 ($M+H$)⁺.

35

EXAMPLE 33

N-Ac-Sar-Gly-Val-D-Pen(SMe)-Thr-Gln-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-D-Pen(SMe) for Fmoc-D-Ile, and Fmoc-Gln(Trt) for Fmoc-Nva in Example 1. After cleavage of the peptide from the resin and removal of the protecting groups the product was precipitated with diethyl ether and filtered. The product was purified by preparative HPLC to provide N-Ac-Sar-Gly-Val-D-Pen(SMe)-Thr-Gln-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 21.5 minutes (using a C-18 column and a solvent system increasing in gradient over 33 minutes from 10% to 40% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1055 (M+H)⁺.

EXAMPLE 34

N-Ac-Sar-Gly-Gln-D-Pen(SMe)-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-Gln(Trt) for Fmoc-Val, and Fmoc-D-Pen(SMe) for Fmoc-D-Ile in Example 1. After cleavage of the peptide from the resin and removal of the protecting groups the product was precipitated with diethyl ether and filtered. The product was purified by preparative HPLC to provide N-Ac-Sar-Gly-Gln-D-Pen(SMe)-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 19.5 minutes (using a C-18 column and a solvent system increasing in gradient over 33 minutes from 10% to 40% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1055 (M+H)⁺.

EXAMPLE 35

N-Ac-Sar-Gly-Asn-D-Pen(SMe)-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid, Fmoc-Asn(Trt) for Fmoc-Val, and Fmoc-D-Pen(SMe) for Fmoc-D-Ile. After cleavage of the peptide from the resin and removal of the protecting groups the product was precipitated with diethyl ether and filtered. The product was purified by preparative HPLC to provide N-Ac-Sar-Gly-Asn-D-Pen(SMe)-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 21.0 minutes (using a C-18 column and a solvent system increasing in gradient over 33 minutes from 10% to 40% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1041 (M+H)⁺.

EXAMPLE 36

N-Ac-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Orn-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid and Fmoc-Orn(N-delta-Boc) for Fmoc-Arg(Pmc) in Example 1. After cleavage of the peptide from the resin and removal of the protecting groups the product was precipitated with

diethyl ether and filtered. The product was purified by preparative HPLC to provide N-Ac-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Orn-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 3.113 minutes (using a C-18 column and a solvent system increasing in gradient over 33 minutes from 10% to 40% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 952 (M+H)⁺; Amino Acid Anal.: 0.94 Sar; 1.09 Gly; 1.10 Val; 1.86 Ile; 0.65 Thr; 0.95 Nva; 0.98 Orn; 1.03 Pro.

EXAMPLE 37

N-Ac-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Gln-ProNHCH₂CH₃

The desired product was prepared by substituting acetic acid for N-acetylnipecotinic acid and Fmoc-Gln(Trt) for Fmoc-Arg(Pmc) in Example 1. After cleavage of the peptide from the resin and removal of the protecting groups the product was precipitated with diethyl ether and filtered. The product was purified by preparative HPLC to provide N-Ac-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Gln-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 3.113 minutes (using a C-18 column and a solvent system increasing in gradient over 33 minutes from 10% to 40% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 966 (M+H)⁺; Amino Acid Anal.: 0.94 Sar; 0.99 Gly; 1.02 Val; 1.87 Ile; 0.65 Thr; 0.97 Nva; 0.52 Glu; 1.12 Pro.

EXAMPLE 38

N-Ac-Sar-Gly-Val-D-Ile-Thr(OAc)-Orn(N-delta-Ac)-Ile-Arg-ProNHCH₂CH₃

A solution of N-Ac-Sar-Gly-Val-D-Ile-Thr-Orn-Ile-Arg-ProNHCH₂CH₃ (70 mg, prepared by substituting acetic acid for N-acetylnipecotinic acid and Fmoc-Orn(N-delta-Boc) for Fmoc-Nva in Example 1) was treated with (1:1:8) acetic anhydride/pyridine/DMF (3 mL) for about 42 hours. The solvent and the excess reagent were removed under vacuum and the residue was precipitated with diethyl ether. The precipitate was filtered to provide N-Ac-Sar-Gly-Val-D-Ile-Thr(OAc)-Orn(N-delta-Ac)-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 3.05 minutes (using a C-18 column and a solvent system increasing in gradient over 33 minutes from 10% to 40% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1093 (M+H); Amino Acid Anal.: 0.71 Sar; 0.96 Gly; 0.99 Val; 2.06 Ile; 0.62 Thr; 1.09 Orn; 1.00 Arg; 1.13 Pro.

EXAMPLE 39

N-MePro-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar and omitting the N-acetylnipecotinic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was

purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; MS (ESI) m/e 992 (M+H)⁺.

5

EXAMPLE 40

N-MePro-Gly-Val-D-alloIle-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar and Fmoc-D-alloIle for Fmoc-D-Ile and omitting the N-acetylnipecotic acid coupling in Example 1.

10 Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Me-Pro-Gly-Val-D-alloIle-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt, R_t = 2.977 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 95% acetonitrile/water containing 10 mM ammonium acetate); MS (ESI) m/e 992 (M+H)⁺; Amino Acid Anal.: 1.00 Pro; 0.97 Arg; 2.07 Ile; 1.00 Nva; 0.54 Thr; 0.99 Val; 0.97 Gly.

EXAMPLE 41

N-MePro-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar and Fmoc-D-Leu for Fmoc-D-Ile and omitting the N-acetylnipecotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Me-Pro-Gly-Val-D-Leu-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt, R_t = 3.15 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 95% acetonitrile/water containing 10 mM ammonium acetate); MS (ESI) m/e 992 (M+H)⁺; Amino Acid Anal.: 1.04 Pro; 1.01 Arg; 1.93 Ile; 1.03 Nva; 0.54 Thr; 1.02 Val; 0.98 Gly.

EXAMPLE 42

N-MePro-Gly-Val-D-Ile-Thr-Gln-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar and Fmoc-Gln(Trt) for Fmoc-Nva and omitting the N-acetylnipecotic acid coupling in Example 1.

Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient from 5% to 100% acetonitrile/water containing 0.01% TFA over a period of 50 min. The pure fractions were lyophilized to provide N-MePro-Gly-Val-D-Ile-Thr-Gln-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 2.718 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1021 (M+H)⁺; Amino Acid Anal.: 1.05 Pro; 1.07 Arg; 1.96 Ile; 0.92 Val; 0.94 Glu; 0.36 Thr; 1.05 Gly.

EXAMPLE 43

N-MePro-Gly-Gln-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar and Fmoc-Gln(Trt) for Fmoc-Val and omitting the N-acetylnipicotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Gln-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 2.083 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1021 (M+H)⁺; Amino Acid Anal.: 1.00 Pro; 1.03 Arg; 2.07 Ile; 1.01 Nva; 0.93 Glu; 0.43 Thr; 0.95 Gly.

EXAMPLE 44

N-MePro-Gly-Val-D-Ile-Thr-Nva-D-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar and Fmoc-D-Ile for Fmoc-Ile and omitting the N-acetylnipicotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Val-D-Ile-Thr-Nva-D-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 3.06 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 992 (M+H)⁺; Amino Acid Anal.: 1.04 Pro; 1.05 Arg; 2.01 Ile; 1.01 Nva; 0.95 Val; 0.45 Thr; 0.95 Gly.

EXAMPLE 45

N-MePro-Gly-Gln-D-Ile-Thr-Nva-D-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar, Fmoc-Gln(Trt) for Fmoc-Val, and Fmoc-D-Ile for Fmoc-Ile and omitting the N-acetylnipecotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Gln-D-Ile-Thr-Nva-D-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 2.331 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1021 (M+H)⁺; Amino Acid Anal.: 1.02 Pro; 1.03 Arg; 2.10 Ile; 1.00 Nva; 0.92 Glu; 0.47 Thr; 0.93 Gly.

EXAMPLE 46

N-MePro-Gly-Val-D-Ile-alloThr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting Fmoc-alloThr(OtBu) for Fmoc-Thr(OtBu) in Example 39. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Val-D-Ile-alloThr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 2.809 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 992 (M+H)⁺; Amino Acid Anal.: 1.03 Pro; 1.07 Arg; 2.00 Ile; 1.01 Nva; 0.96 Val; 0.50 Thr; 0.94 Gly.

EXAMPLE 47

N-MePro-Gly-Gln-D-Ile-Thr-Nva-Ile-Arg-Pro-D-AlaNH₂

The desired product was prepared by the procedure described in Example 1 with the following modifications: N-MePro was substituted for Fmoc-Sar, Fmoc-Gln(Trt) was substituted for Fmoc-Val, and Fmoc-D-Ala-Sieber amide resin was substituted for Fmoc-Pro-Sieber ethylamide resin. In addition, the N-acetylnipecotic acid coupling was omitted and a coupling with Fmoc-Pro was added prior to the coupling with Fmoc-Arg(Pmc). Upon

completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Gln-D-Ile-Thr-Nva-Ile-Arg-Pro-D-AlaNH₂ as the trifluoroacetate salt; R_t = 1.75 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1064 (M+H)⁺; Amino Acid Anal.: 1.05 Ala; 1.04 Pro; 0.99 Arg; 2.07 Ile; 1.01 Nva; 0.87 Glu; 0.42 Thr; 0.96 Gly.

EXAMPLE 48

N-MePro-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-Pro-D-AlaNH₂

The desired product was prepared by substituting N-MePro for Fmoc-Sar and Fmoc-D-Ala-Sieber amide resin for Fmoc-Pro-Sieber ethylamide resin in Example 1. In addition, a coupling with Fmoc-Pro was added prior to the coupling with Fmoc-Arg(Pmc) and the coupling with N-acetylنيpecotic acid was omitted. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-Pro-D-AlaNH₂ as the trifluoroacetate salt; R_t = 2.695 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1035 (M+H)⁺; Amino Acid Anal.: 1.09 Ala; 1.08 Pro; 0.96 Arg; 2.01 Ile; 1.02 Nva; 0.91 Val; 0.40 Thr; 0.94 Gly.

EXAMPLE 49

N-MePro-Gly-Gln-D-alloIle-Thr-Nva-Ile-Arg-Pro-D-AlaNH₂

The desired product was prepared by substituting N-MePro for Fmoc-Sar, Fmoc-Gln(Trt) for Fmoc-Val, Fmoc-D-alloIle for Fmoc-D-Ile, and Fmoc-D-Ala-Sieber amide resin for Fmoc-Pro-Sieber ethylamide resin in Example 1. In addition, a coupling with Fmoc-Pro was added prior to the coupling with Fmoc-Arg(Pmc) and the coupling with N-acetylنيpecotic acid was omitted. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-

MePro-Gly-Gln-D-alloIle-Thr-Nva-Ile-Arg-Pro-D-AlaNH₂ as the trifluoroacetate salt; R_t = 1.708 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1064 (M+H)⁺; Amino Acid Anal.: 1.00 Ala; 1.00 Pro; 0.96 Arg; 2.20 Ile; 1.00 Nva; 0.90 Glu; 0.44 Thr; 0.94 Gly.

EXAMPLE 50

N-MePro-Gly-Ile-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar and Fmoc-Ile for Fmoc-Val and omitting the N-acetylnipecotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Ile-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 3.092 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1006 (M+H)⁺; Amino Acid Anal.: 0.99 Pro; 1.06 Arg; 3.02 Ile; 1.02 Nva; 0.41 Thr; 0.96 Gly.

EXAMPLE 51

N-MePro-Gly-Val-D-alloIle-Ser-Ser-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar, Fmoc-D-alloIle for Fmoc-D-Ile, and Fmoc-Ser(OtBu) for both Fmoc-Thr(OtBu) and Fmoc-Nva and omitting the N-acetylnipecotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Val-D-alloIle-Ser-Ser-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 2.474 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 966 (M+H)⁺; Amino Acid Anal.: 1.04 Pro; 1.03 Arg; 1.03 alloIle; 0.98 Ile; 1.03 Nva; 0.42 Ser; 0.95 Gly.

EXAMPLE 52

N-MePro-Gly-Asn-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar and Fmoc-Asn(Trt) for Fmoc-Val and omitting the N-acetylnipecotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Asn-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 1.975 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1007 (M+H)⁺; Amino Acid Anal.: 1.01 Pro; 1.04 Arg; 2.07 Ile; 0.99 Nva; 0.40 Thr; 0.96 Asp; 0.92 Gly.

EXAMPLE 53

N-MePro-Gln-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar and Fmoc-Gln(Trt) for Fmoc-Gly and omitting the N-acetylnipecotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gln-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 2.73 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1063 (M+H)⁺; Amino Acid Anal.: 1.04 Pro; 1.04 Arg; 2.00 Ile; 1.02 Nva; 0.50 Thr; 0.98 Val; 0.92 Glu.

EXAMPLE 54

N-MePro-Gln-Val-D-Ile-Thr-Nva-Ile-Arg-Pro-D-AlaNH₂

The desired product was prepared by substituting N-MePro for Fmoc-Sar, Fmoc-Gln(Trt) for Fmoc-Gly, and Fmoc-D-Ala-Sieber amide resin for Fmoc-Pro-Sieber ethylamide resin in Example 1. In addition, a coupling with Fmoc-Pro was added prior to the coupling with Fmoc-Arg(Pmc) and the coupling with N-acetylnipecotic acid was omitted. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gln-Val-D-Ile-Thr-Nva-Ile-Arg-Pro-D-

AlaNH₂ as the trifluoroacetate salt; R_t = 2.619 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1106 (M+H)⁺; Amino Acid Anal.: 1.08 Ala; 1.12 Pro; 1.06 Arg; 2.06 Ile; 1.02 Nva; 0.44 Thr; 0.90 Val; 0.77 Glu.

5

EXAMPLE 55

N-MePro-Gly-Val-D-Ile-alloThr-Gln-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar, Fmoc-alloThr(OtBu) for Fmoc-Thr(OtBu), and Fmoc-Gln(Trt) for Fmoc-Nva and omitting the N-acetyl nipecotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Val-D-Ile-alloThr-Gln-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 2.37 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1021 (M+H)⁺.

03015956 "09272601

EXAMPLE 56

N-MePro-Gly-Val-D-Ile-alloThr-Nva-Ile-Arg-Pro-D-AlaNH₂

The desired product was prepared by substituting N-MePro for Fmoc-Sar, Fmoc-alloThr(OtBu) for Fmoc-Thr(OtBu), and Fmoc-D-Ala-Siebert amide resin for Fmoc-Pro-Siebert ethylamide resin in Example 1. In addition, a coupling with Fmoc-Pro was added prior to the coupling with Fmoc-Arg(Pmc) and the coupling with N-acetyl nipecotic acid was omitted. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient from 5% to 100% acetonitrile/water containing 0.01% TFA over a period of 50 min. The pure fractions were lyophilized to provide N-MePro-Gly-Val-D-Ile-alloThr-Nva-Ile-Arg-Pro-D-AlaNH₂ as the trifluoroacetate salt; R_t = 2.49 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1035 (M+H)⁺.

35

EXAMPLE 57

N-MePro-Gly-Val-D-Leu-Ser-Nva-Ile-Arg-Pro-D-AlaNH₂

The desired product was prepared by substituting N-MePro for Fmoc-Sar, Fmoc-D-Leu for Fmoc-D-Ile, Fmoc-Ser(OtBu) for Fmoc-Thr(OtBu), and Fmoc-D-Ala-Sieber amide resin for Fmoc-Pro-Sieber ethylamide resin in Example 1. In addition, a coupling with Fmoc-Pro was added prior to the coupling with Fmoc-Arg(Pmc) and the coupling with N-acetylnicopetic acid was omitted. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Val-D-Leu-Ser-Nva-Ile-Arg-Pro-D-AlaNH₂ as the trifluoroacetate salt; R_t = 2.802 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1021 (M+H)⁺.

EXAMPLE 58

N-MePro-Gly-Val-D-Ile-alloThr-Ser-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar, Fmoc-alloThr(tBu) for Fmoc-Thr(tBu), and Fmoc-Ser(OtBu) for Fmoc-Nva and omitting the N-acetylnipectic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Val-D-Ile-alloThr-Ser-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 2.452 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 980 (M+H)⁺.

EXAMPLE 59

N-MePro-Gly-Val-D-Ile-Thr-alloThr-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting N-MePro for Fmoc-Sar, Fmoc-alloThr(OtBu) for Fmoc-Nva, and omitting the N-acetylnipectic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-MePro-Gly-Val-D-Ile-Thr-alloThr-

Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt; R_t = 2.452 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 994 (M+H)⁺.

5

EXAMPLE 60

N-Ac-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-Pro-NMe-D-AlaNH₂

The desired product was prepared by substituting acetic acid for N-acetylnipecotic acid and Fmoc-N-Me-D-Ala-Sieber amide resin for Fmoc-Pro-Sieber ethylamide resin. In addition, a coupling with Fmoc-Pro was added prior to the coupling with Fmoc-Arg(Pmc). Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by preparative HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-Pro-N-Me-D-AlaNH₂ as the trifluoroacetate salt; R_t = 2.53 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 10% to 95% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1051.8 (M+H)⁺.

0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

EXAMPLE 61

N-[(N-acetylazetidine-2-carbonyl)]-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting Fmoc-azetidine-2-carboxylic acid for N-acetylnipecotic acid in Example 1 and adding a coupling with acetic acid after the coupling with the Fmoc-azetidine-2-carboxylic acid. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-[(N-acetylazetidine-2-carbonyl)]-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 2.87 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1077 (M+H)⁺; Amino Acid Anal.: 1.02 Sar; 1.03 Gly; 0.97 Val; 2.11 Ile; 0.55 Thr; 1.01 Nva; 1.05 Arg; 1.01 Pro.

EXAMPLE 62

N-[(N-acetylazetidine-3-carbonyl)]-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product was prepared by substituting Fmoc-azetidine-3-carboxylic acid for N-acetylnipecotic acid and adding a coupling with acetic acid after the coupling with

Fmoc-azetidine-3-carboxylic acid in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-[(N-acetylazetidine-3-carbonyl)]-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt: R_t = 2.87 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1077 (M+H)⁺; Amino Acid Anal.: 1.00 Sar; 1.02 Gly; 1.02 Val; 2.04 Ile; 0.49 Thr; 0.98 Nva; 1.10 Arg; 1.03 Pro.

EXAMPLE 63

N-Ac-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-Pro-D-Lys(Ac)NH₂

The desired product was prepared by substituting Fmoc-D-Lys(Ac)-Sieber amide resin for Fmoc-Pro-Sieber ethylamide resin and acetic acid for N-acetylnipecotic acid in Example 1. Upon completion of the synthesis, cleavage of the peptide from the resin, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide was obtained. This was purified by HPLC using a C-18 column and a solvent system increasing in gradient over 50 minutes from 5% to 100% acetonitrile/water containing 0.01% TFA. The pure fractions were lyophilized to provide N-Ac-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-Pro-D-Lys(Ac)NH₂ as the trifluoroacetate salt: R_t = 2.84 minutes (using a C-18 column and a solvent system increasing in gradient over 10 minutes from 20% to 80% acetonitrile/water containing 0.01% TFA); MS (ESI) m/e 1136.8 (M+H)⁺; Amino Acid Anal.: 0.97 Sar; 1.01 Gly; 1.03 Val; 2.05 Ile; 0.55 Thr; 1.01 Nva; 0.99 Arg; 0.98 Pro.

EXAMPLE 64

N-MePro-Gly-Val-D-Ile-alloThr-Nva-Pro-Arg-ProNHCH₂CH₃

The desired product can be prepared by substituting N-MePro for Fmoc-Sar, Fmoc-alloThr(OrBu) for Fmoc-Thr(OrBu), and Fmoc-Pro for Fmoc-Ile, and omitting the N-acetylnipecotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide can be obtained.

EXAMPLE 65

N-MePro-Gly-Val-D-alloIle-Thr-Trp-Ile-Arg-ProNHCH₂CH₃

The desired product can be prepared by substituting N-MePro for Fmoc-Sar, Fmoc-D-alloIle for Fmoc-D-Ile, and Fmoc-Trp(Boc) for Fmoc-Nva and omitting the N-

acetylnipecotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide can be obtained.

EXAMPLE 66

N-MePro-Gly-Val-D-Ile-Thr-Gln-D-Ile-Arg-ProNHCH₂CH₃

The desired product can be prepared by substituting N-MePro for Fmoc-Sar, Fmoc-Gln(Trt) for Fmoc-Nva and Fmoc-D-Ile for Fmoc-Ile and omitting the N-acetylnipecotic acid coupling in Example 1. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide can be obtained.

EXAMPLE 67

N-Ac-N-MeNva-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

In the reaction vessel of an Applied Biosystems 433A peptide synthesizer is placed Fmoc-Pro-Sieber ethylamide resin (0.1 mM). Cartridges of 1mM amino acids are sequentially loaded. Using the Fastmoc 0.1 with previous peak monitoring the following protocol is used:

- (1) Solvate resin with NMP for about 5 minutes;
- (2) Wash resin with NMP for about 5 minutes;
- (3) Remove Fmoc group using 50% piperidine solution in NMP for 5 minutes, wash resin, and repeat the sequence 3 to 4 times;
- (4) Activate protected amino acid with 1 mM of 0.5M HATU in DMF;
- (5) Add Activated protected amino acid to reaction vessel followed by 1 mM of 2M diisopropylamine in NMP;
- (6) Couple protected amino acid for 20 minutes;
- (7) Wash resin and remove protecting group with 50% piperidine in NMP.

The protected amino acids can be coupled to the resin in the following order:

Amino acid	Coupling time
1. Fmoc-Arg(Pmc)	20 minutes
2. Fmoc-Ile	20 minutes
3. Fmoc-Nva	20 minutes
4. Fmoc-Thr(OtBu)	20 minutes
5. Fmoc-D-Ile	20 minutes
6. Fmoc-Val	20 minutes

7. Fmoc-Gly	20 minutes
8. Fmoc-N-MeNva	20 minutes
9. acetic acid	20 minutes

Upon completion of the synthesis the resin-bound peptide can be washed with methanol, dried under vacuum, and treated with (95:5) TFA/water (3 mL) at room temperature for 18 hours. The resin is filtered and washed with methanol. The filtrates and the washes are combined and concentrated. The residue is treated with diethyl ether and the precipitate is filtered to provide the crude peptide. This can be purified by preparative HPLC, then lyophilized to provide N-Ac-N-MeNva-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃ as the trifluoroacetate salt.

EXAMPLE 68

N-Ac-N-MeThr(Bzl)-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-ProNHCH₂CH₃

The desired product can be prepared by substituting Fmoc-N-MeThr(OBzl) for Fmoc-N-MeNva in Example 67. Upon completion of the synthesis, cleavage of the resin-bound peptide, removal of the protecting groups, precipitation with diethyl ether, and filtration the crude peptide can be obtained.

It will be evident to one skilled in the art that the present invention is not limited to the foregoing illustrative examples, and that it can be embodied in other specific forms without departing from the essential attributes thereof. It is therefore desired that the examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing examples, and all changes which come within the meaning and range of equivalency of the claims and therefore intended to be embraced therein.